Association of angiotensin converting enzyme and angiotensin II type 1 receptor genotypes with left ventricular function and mass in patients with angiographically normal coronary arteries

Martial Hamon, Carole Amant, Christophe Bauters, Florence Richard, Nicole Helbecque, Eugène Mc Fadden, Jean-Marc Lablanche, Michel Bertrand, Philippe Amouyel

Abstract

***Objective*—To analyse the potential association of the angiotensin converting enzyme (ACE) and angiotensin II type 1 receptor (AT1R) gene polymorphisms on left ventricular function and mass in patients with normal coronary arteries.**

***Design*—Consecutive sample.**

***Setting*—University hospital.**

***Subjects*—141 consecutive white patients referred for coronary angiography and with angiographically normal coronary arteries. Patients with valvar diseases, cardiomyopathies, or a history of myocardial infarction were excluded.**

***Main outcome measures*—Left ventricular variables were measured for all patients. The ACE and AT1R genotypes were determined with a polymerase chain reaction based protocol using DNA prepared from white blood cells. A general linear model was used to compare data according to the ACE and to the AT1R genotypes.**

***Results*—A strong association was observed between left ventricular mass and systemic hypertension (mean (SD) hypertension: 114 (31) g/m^2; no hypertension 98 (23) g/m^2; $P < 0.003$). However, no influence of ACE and AT1R polymorphisms on left ventricular mass was found, regardless of systemic hypertension. The subjects homozygous for the AT1R CC mutation had a significantly lower ejection fraction than those with allele A (AC+AA) (mean (SD) 62(12)% and 68(10)%, respectively, $P < 0.05$). No synergistic interaction of ACE and AT1R gene polymorphisms on left ventricular function and mass was found.**

***Conclusions*—These data do not support an association of the ACE and AT1R genotypes on left ventricular hypertrophy in white patients with normal coronary arteries.**

Service de Cardiologie B et Hémodynamique, Hôpital Cardiologique, CHRU de Lille, France
M Hamon
C Bauters
E Mc Fadden
J-M Lablanche
M Bertrand

Service d'Epidémiologie et de Santé Publique/ INSERM CJF 95–05, Institut Pasteur de Lille, Lille, France
C Amant
F Richard
N Helbecque
P Amouyel

Correspondence to:
Dr Martial Hamon, Service de Cardiologie B, Hôpital Cardiologique, Boulevard du Professeur J Leclercq, 59037 Lille, France.

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It has been suggested that the components of the renin-angiotensin system play a major role in the pathogenesis of a wide variety of cardiovascular diseases. The deletion polymorphism of the angiotensin converting enzyme (ACE) gene, associated with increased levels of the plasma enzyme, is a risk factor for myocardial infarction.[1 2] Another genetic polymorphism, the A/C mutation of the angiotensin II type 1 receptor (AT1R) gene, interacts with the DD genotype to increase the risk of myocardial infarction synergistically.[3] Two studies have reported an influence of ACE polymorphism on left ventricular hypertrophy[4 5]: the DD genotype was associated with increased left ventricular mass in a population based study[4] and in an outpatient clinic study.[5] However, two other studies[6 7] were not able to detect any influence of ACE gene polymorphism on left ventricular hypertrophy. Differences in population selection, in the techniques used to assess left ventricular hypertrophy, or interactions with other genetic polymorphisms of the renin-angiotensin system such as the AT1R polymorphism, may account for these discrepancies. In an attempt to reduce the influence of confounding factors such as coronary artery disease, we analysed the effect of the ACE DD genotype on left ventricular mass and function in a group of patients with angiographically normal coronary arteries. We also explored the effect of the (A/C) AT1R genotype.

Methods

PATIENTS

Between March 1993 and April 1994, 141 consecutive patients with normal coronary arteries were included in this study, after informed consent was obtained from each patient. All patients were referred to our institution for coronary angiography because of chest pain associated with risk factors for coronary artery disease or with a positive exercise test. Patients with valvar diseases, cardiomyopathies, or a history of myocardial infarction were excluded. All patients underwent left ventricular angiography followed by coronary angiography in conjunction with a provocative test to exclude coronary spasm. Blood samples for subsequent genetic analysis were obtained at the time of cardiac catheterisation.

LEFT VENTRICULAR FUNCTION AND MASS

Opacification of the left ventricular chamber was performed using an 8F pigtail catheter to deliver 0·5 ml/kg of contrast media (Radioselectan) at a rate of 13 ml/s in the right oblique anterior projection. Magnification correction was achieved by filming a circular disk

Table 1 Influence of sex and hypertension on left ventricular variables

Left ventricular variables	*Men (n = 95)*	*Women (n = 46)*	*With hypertension (n = 65)*	*Without hypertension (n = 76)*
Ejection fraction (%)	67 (10)	69 (11)	67 (10)	68 (10)
End diastolic volume (ml/m²)	82 (18)	81 (22)	82 (20)	81 (19)
End systolic volume (ml/m²)	28 (11)	26 (17)	28 (14)	26 (13)
Stroke volume (ml/m²)	54 (13)	54 (13)	54 (13)	55 (13)
Left ventricular mass (g/m²)	108 (28)	99 (25)*	114 (31)	98 (23)†

Values are mean (SD), *P < 0·07, †P < 0·003.

of known diameter at mid-chest level. Ventriculograms were recorded on 35 mm cinefilm, allowing projection for subsequent analysis. All measurements were performed by a trained technician. After tracing the end diastolic and end systolic left ventricular outlines and measuring the end diastolic thickness of the left ventricular free wall, the left ventricular chamber and wall volumes were calculated as previously described.[8] Left ventricular mass was then calculated according to the specific gravity of heart muscle[9]; this method has previously been validated by necropsy studies (correlation coefficient $r = 0{\cdot}97$, SD = 32 g).[10] Left ventricular stroke volume was calculated as the difference between end diastolic volume and end systolic volume. The ejection fraction is the ratio between stroke volume and end diastolic volume. An adjustment for body surface area was performed.

GENETIC STUDY

Blood samples were collected in Vacutainer tubes containing EDTA anticoagulant. Genomic DNA was prepared from white blood cells as previously described.[11] The ACE gene fragment containing the insertion/deletion sequence[12] and AT1R gene fragment containing A/C mutation[13] were amplified with a Perkin Elmer DNA thermal cycler and *Thermus aquaticus* DNA polymerase (Amersham) and detected as previously described,[14] except for addition of dimethyl sulphoxide (DMSO) to enhance amplification of the ACE I allele.[15] For technical reasons, the AT1R genotypes were not available for seven patients.

STATISTICAL ANALYSIS

Statistical analyses were performed with the SAS Software release 6·10 (SAS Institute, Cary, North Carolina, USA). Means and standard deviations of quantitative data were calculated. Quantitative data were compared with a general linear model according to the ACE (DD, ID, and II) and to the AT1R (CC, AC, and AA) genotypes. Qualitative data were tested using Pearson's χ^2. All statistical tests were adjusted for age, sex, body mass index (BMI, kg/m²), family history of myocardial infarction, hypotensive drug consumption, and history of hypertension.

Results

The mean (SD) age of the 141 subjects (95 males and 46 females) was 53 (11) years (men, 52 (10); women, 56 (10)). The mean (SD) BMI of the sample was 27·5 (4·7) kg/m² (men, 27·4 (4·1) kg/m²; women, 27·7 (5·8) kg/m²). Forty three per cent had a family history of coronary artery disease (men, 37%; women, 56%), 31% were smokers (men, 41%; women, 8%) and 11% were diabetics (men, 8%; women, 17%). Systemic hypertension (defined as a systolic blood pressure $\geqslant$ 160 mm Hg, and/or a diastolic blood pressure $\geqslant$ 95 mm Hg, and/or antihypertensive drug treatment) was observed for 46% (men, 45%; women, 47%). ACE inhibitors were taken by 12% of patients and prescription of calcium inhibitors and β blockers was observed in 28% and 21% of cases, respectively. The overall population had a left ventricular function within normal limits (mean (SD) ejection fraction 67 (10)%), and a mean (SD) left ventricular mass of 105 (27) g/m². The influence of sex and hypertension on left ventricular variables is shown in table 1. No major significant difference was observed between males and females except for a trend towards a higher left ventricular mass in males (P < 0·07). As expected, a strong association was observed between left ventricular mass and the presence of systemic hypertension (P < 0·003). Other left ventricular measurements including left ventricular volumes and ejection fraction were not influenced by hypertension.

In our sample, the genotype DD, ID, and II distributions for the ACE polymorphism were 33%, 47%, and 20%, respectively. The genotypes CC, AC, and AA of AT1R were 9%, 44%, and 47%, respectively. Both distributions were in Hardy-Weinberg equilibrium. No sex differences were observed.

Left ventricular volumes, ejection fraction, and mass as a function of ACE and AT1R genotypes are given in table 2, according to the presence or absence of hypertension. No

Table 2 Influence of ACE and AT1R genotypes on left ventricular variables

	ACE genotype			*AT1R genotype*		
	DD	*ID*	*II*	*CC*	*AC*	*AA*
A With hypertension	(n = 20)	(n = 31)	(n = 14)	(n = 7)	(n = 24)	(n = 29)
Ejection fraction (%)	69 (8)	66 (11)	64 (12)	62 (14)	66 (11)	67 (8)
End diastolic volume (ml/m²)	73 (15)	83 (7)	93 (22)	81 (14)	90 (26)	77 (14)
End systolic volume (ml/m²)	23 (9)	29 (13)	34 (17)	31 (12)	32 (18)	25 (9)
Stroke volume(ml/m²)	50 (10)	54 (13)	59 (15)	50 (13)	58 (14)	51 (11)
Left ventricular mass(g/m²)	109 (32)	115 (32)	118 (26)	118 (37)	123 (28)	110 (30)
B Without hypertension	(n = 27)	(n = 35)	(n = 14)	(n = 5)	(n = 35)	(n = 34)
Ejection fraction (%)	67 (11)	68 (10)	70 (12)	63 (10)	70 (10)	68 (10)
End diastolic volume (ml/m²)	82 (21)	82 (15)	77 (22)	81 (28)	82 (19)	80 (17)
End systolic volume (ml/m²)	28 (14)	27 (13)	24 (13)	32 (21)	25 (14)	26 (10)
Stroke volume (ml/m²)	54 (14)	56 (12)	53 (15)	49 (8)	57 (13)	54 (14)
Left ventricular mass (g/m²)	99 (23)	98 (22)	98 (25)	108 (15)	94 (25)	102 (21)

Values are mean (SD).

Table 3 Interaction of ACE and AT1R gene polymorphisms on left ventricular mass (g/m²)

	AT1R genotype	
	AC+CC	AA
ACE genotype		
DD	108 (29) (n = 50)	106 (26) (n = 40)
ID+II	105 (31) (n = 21)	104 (25) (n = 23)

Values are mean (SD).

significant influence on left ventricular variables was observed for either polymorphism. This absence of association persisted despite adjustment for age, sex, BMI, family history of myocardial infarction, hypotensive drug consumption, and hypertension. However, the subjects homozygous for the AT1R CC mutation had a lower ejection fraction compared with allele A bearers (AC + AA), both in subjects with and without hypertension. In the whole sample, this effect reached statistical significance (mean (SD) CC bearers 62 (12)%, AC + AA bearers 68(10)%, $P < 0.05$).

As described for the occurrence of myocardial infarction,[3] we investigated a possible synergistic interaction between ACE and AT1R gene polymorphisms on left ventricular mass (table 3). In our study, the left ventricular mass in the four genetic subgroups, defined as a recessive effect of ACE D allele (that is, DD *v* ID+II) and a dominant effect of AT1R C allele (that is, CC + AC *v* AA), did not differ.

Discussion

No influence of the deletion polymorphism of the ACE gene or the mutation A/C of the AT1R gene could be detected on left ventricular hypertrophy in subjects with angiographically normal coronary arteries. A reduced ejection fraction was only observed for homozygous AT1R CC subjects.

Angiotensin II is implicated in the modulation of cardiac growth,[16] and converting enzyme inhibitors have been recognised as one of the most effective means of causing regression or preventing left ventricular hypertrophy.[17] An increased expression of ACE messenger RNA and ACE activity in the hypertrophied ventricle has already been reported.[18] Most of the trophic effects of angiotensin II on cardiomyocytes seem to be mediated through the activation of AT1 receptors.[19] The recent cloning of the ACE and AT1R genes has allowed the identification of gene polymorphisms that affect the function of the renin-angiotensin system.[20,21] The I/D polymorphism of ACE gene is a determinant of the level of serum and cellular ACE activity.[1,22] The DD genotype that is associated with the highest ACE levels is a risk factor for myocardial infarction[2] as well as for dilated and hypertrophic cardiomyopathies.[23,24] Recently, Tiret *et al* have shown that the association between DD ACE genotypes and myocardial infarction was increased in a subset of patients also carriers of the AT1R C allele.[3]

Conflicting results have been reported regarding a possible association of the DD genotype with left ventricular hypertrophy.[4-7] While Schunkert *et al*[4] and Iwai *et al*[5] found an association between the ACE DD genotype and left ventricular hypertrophy, two other studies[6,7] did not find any association. These discrepancies may be due to different techniques for assessing left ventricular hypertrophy and to differences between populations. Schunkert *et al*[4] used electrocardiographic criteria for assessing left ventricular hypertrophy in a population based random sample of 1428 patients, 45 to 59 years of age. These investigators found an increased risk of left ventricular hypertrophy among normotensive men homozygous for the D allele of ACE. A possible limitation of this study relates to the use of electrocardiographic criteria for the identification of left ventricular hypertrophy. Electrocardiographic voltage criteria are poorly correlated with left ventricular mass assessed by echocardiography or measured at necropsy (correlation coefficient *r* ranged from 0·40 to 0·55).[25-27] In the study by Iwai *et al*,[5] left ventricular mass was assessed by echocardiography, which has been shown to be highly correlated with necropsy analysis (correlation coefficient $r = 0.96$, SD = 29 g).[28] However, this study was performed in 141 Japanese patients aged 55 (10) years with various cardiovascular diseases and with a different ethnic background. The negative study of Kupari *et al*,[6] with left ventricular mass assessed by echocardiography, was performed in a random sample of the general population, aged 36 to 37 years, free from clinical heart disease; the other negative study of Lindpainter *et al*[7] was also performed with echocardiography in a large population based study. Our series, which includes only patients with normal coronary arteries and without coronary spasm, was designed to study the impact of the ACE DD genotype on left ventricular hypertrophy independently of coronary artery disease. Our results are in agreement with those of Kupari *et al*[6] and Lindpainter *et al*,[7] showing no significant influence of the ACE genotype on left ventricular function or mass. Moreover, we found no effect of the AT1R genotype on left ventricular hypertrophy and no interaction between the ACE DD genotype and AT1R C allele. Finally, although left ventricular mass was significantly increased in the subgroup of patients who had a history of hypertension, there was no effect of ACE or AT1R gene polymorphisms on left ventricular hypertrophy in either group, showing that left ventricular hypertrophy was not affected by these polymorphisms even in the presence of hypertension.

Taken together, these data suggest that ACE and AT1R gene polymorphisms have no or minimal impact on left ventricular function and mass in white patients without coronary artery disease, valvar heart disease, or cardiomyopathy. Because of the relatively small size of our study group, compared to the number of subjects in Schunkert's report,[4] we calculated the statistical power of our study. Given the number of subjects and the standard deviation observed in our study, the power was 0·88

to detect a difference of 20 g/m^2 between the adjusted left ventricular mass in the II and DD genotypes (as observed in Iwai's report[5]) at an α level of 0·05. This statistical power calculation showed that the probability of failing to detect an association in our sample between the ACE or AT1R genotypes and left ventricular mass was low. Our results are not in conflict with previous studies showing an influence of the DD genotype in dilated or ischaemic cardiomyopathies,[23 24] because we excluded patients with evidence of myocardial disease. We do not exclude a potentially deleterious effect of the components of the renin-angiotensin system leading to aberrant cardiac remodelling following myocardial injury[29 30]; moreover these results do not preclude the possibility that in the presence of other factors stimulating muscle cell growth these polymorphisms may favour the development of ventricular hypertrophy. Further larger studies are needed to estimate the effect of these genetic polymorphisms on myocardial hypertrophy in the presence of arterial hypertension, and the importance of concomitant treatments.

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